:51:57 OCA PAD INITIATION - PROJECT HEADER INFORMATION 05/06/88

Active

oject #: G-33-A13 Cost share #: Rev #: 0
nter # : Q5250-3A0 Center shr #: OCA file #:
Work type : RES
ntract#: 2 R01 EY01746-13 Mod #: Document : GRANT
ime #: Contract entity: GIT

oprojects ? : N
in project #:

oject unit: CHEM Unit code: 02.010.136
oject director(s):
YU N-T CHEM

onsor/division names: DHHS/PHS/NIH / NATL INSTITUTES OF HEALTH
onsor/division codes: 108 / 001

ard period: 880501 to 890430 (performance) 890731 (reports)

onsor amount	New this change	Total to date
Contract value	231,342.00	231,342.00
Funded	231,342.00	231,342.00
st sharing amount		0.00

es subcontracting plan apply ?: N

tle: COMPARATIVE RAMAN STUDIES OF HUMAN AND ANIMAL LENSES

PROJECT ADMINISTRATION DATA

A contact: E. Faith Gleason 894-4820

ponsor technical contact	Sponsor issuing office
ALPH J.HELMSEN,PH.D, EXMRL PR DIR	FRANCES M. GOFF, GRANTS MGT
301)496-5884	(301)496-5983
ATARACT PROGRAM/NATL EYE INST/NIH	EXTRAMURAL SERVICES BR/NEI/NIH
333 WESTBARD AVE	5333 WESTBARD AVE
ETHESDA, MD 20892	BETHESDA, MD 20892

curity class (U,C,S,TS) : ONR resident rep. is ACO (Y/N): N
fense priority rating : N/A supplemental sheet
uipment title vests with: Sponsor GIT X

ministrative comments -
INITIATION OF 13TH YEAR OF CONTINUING GRANT APPROVED FOR 17 YEARS.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Date 3/7/89

ect No. G-33-A13 Center No. 05250-3A0

ect Director N. T. Yu School/Lab Chem.

sor DHHS/PHS/NIH

ract/Grant No. 2 R01 EY01746-13 GTRC ____ GIT XX

e Contract No. ____

e Comparative Raman Sudies of Human and Animal Lens.

ctive Completion Date 4/30/89 (Performance) 7/31/89 (Reports)

eout Actions Required:

None
Final Invoice or Copy of Last Invoice
Final Report of Inventions and/or Subcontracts
Government Property Inventory & Related Certificate
Classified Material Certificate
Release and Assignment
Other ____

udes Subproject No(s). ____

roject Under Main Project No. ____

inues Project No. G-33-A12 Continued by Project No. G-33-A14

ribution:

Project Director
Administrative Network
Accounting
Procurement/GTRI Supply Services
Research Property Management
Research Security Services

X Reports Coordinator (OCA)
___ GTRC
X Project File
X Contract Support Division (OCA)(2)
___ Other ____

G-33-A13

SECTION IV **PROGRESS REPORT SUMMARY**	GRANT NUMBER EY01746-14	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Yu, Nai-Teng	PERIOD COVERED BY THIS REPORT FROM	THROUGH
APPLICANT ORGANIZATION Georgia Institute of Technology	05/01/88	02/20/89

TITLE OF PROJECT *(Repeat title shown in item 1 on first page)*
Comparative Raman Studies of Human and Animal Lenses
(SEE INSTRUCTIONS)

1. The Plans for the Next Year of Support :

The specific aims for the next year of support are : (1) To complete our current improvement of the servo control interface and the computer software for the surface-scanning laser microprobe imaging system; (2) To obtain the precise distribution profiles of the orange (Emission 591 nm / Excitation 568.2 nm) and the near red fluorophor (Emission 633 nm/Excitation 568.2 nm) in the human lenses of various ages; (3) To develop the techniques of near infrared-excited surface-enhanced Fourier-Transformed Raman spectroscopy for the detection of 3-OH-L-kynurenine-O-b glucoside and its derivatives / reaction products with lens crystallins; (4) To obtain fluorescence-free Raman spectra of human brunescence cataracts using new FT-Raman techniques.

2. Concise Description of the Studies Conducted during the Current Budget Year :

(i) *Distribution of a 488.0-nm-excited fluorophor in the equatorial plane of the human lens*

We have employed the laser microprobe imaging technique (developed in this laboratory) to obtain the first equatorial plane distribution profile of a 488.0-nm-excited fluorophor in the human lens. A 40 x 40 matrix of gridded data points, collected across the frozen sectioned surface, was acquired by the use of a computer-driven X-Y translation stage. The distribution profiles revealed that the 488.0-nm-excited fluorophor is metabolically produced, since its highest concentration is in the area defined behind the iris. A photochemically generated fluorophor should show the maximum concentration in the region near the visual axis. The only significant increase in fluorescence intensity from 71 to 83-year-old lens occurs in the outer cortical region, indicating the metabolic origin.

(ii) *Discovery of two metabolically related fluorophors in the human lens*

Automated laser microprobe imaging revealed the distribution of blue fluorescent 3-OH-L-kynurenine-O-b-glucoside in human lenses from 0.4 to 71 years. A 3-dimensional perspective grid map with fluorescence intensity as the third dimension shows maximum fluorescence in the infant lens nucleus. At 12 years the fluorescence peak is broadened and a toroid-shaped maximum occurs also in the outer cortex, creating a toroid-shaped minimum between the two maxima (see Fig. 1). By 71 years the nuclear maximum is lower but a new (green) fluorophor (excitation 488 nm/emission 530 nm) has appeared as a toroidal maximum in the same location as the blue minimum (see Fig. 2), suggesting the conversion of the blue fluorophor to the unidentified fluorophor.

(iii) *Raman detection of sulfhydryl decrease and water increase along an equatorial diameter of galactose-induced cataract in rat.*

Raman spectroscopy shows that maturation of galactose cataract greatly increase the water signal (at 3417 cm-1) which is correlated with the imbibition of water in the lens. The maximum water/protein ratio (expressed as Raman intensity ratio I 3417/I2936) occurs at the peripheral cortex (i.e., ~ 4.7), which is much higher than the ratios found in Emory cataract (~ 0.3) and in cac-strain mouse cataract (~ 0.5). It is demonstrated that Raman measurement of the intensity ratio I3417/I2936 is a more sensitive way to reflect increase of water in cataract, compared to water concentration (% of wet weight of the lens). The small decrease in the sulfhydryl profile along an equatorial diameter is attributed to the concentration decrease in glutathione. There is no spectroscopic evidence for extensive disulfide bond formation associated with galactosemic cataractogenesis in rat. There is an increase in the tyrosine I832/I858 ratio, indicating a strengthening of the phenolic hydrogen bond. A comparison of the Raman spectra of normal

lenses and mature cataracts reveals no change in conformation of the protein backbone.

(iv) *Disulfide and sulfhydryl in the Emory Mouse Cataract*

Previous studies have shown that UV-exposure enhanced the expected 2SH to SS conversion of normal mouse lenses in the cortical regions. There was essentially no difference in the disulfide profiles of the nuclear region between UV-exposed and control lenses. In this study we compared the visual axis profiles for SH and SS in early Emory mouse cataracts and in clear lenses from age-matched controls. The sulfhydryl profiles show that the SH level of 8.5-month-old Emory mouse lenses is essentially the same as that of the controls. Likewise, the disulfide profiles show no significant difference. Thus, it is clear that Emory mouse lenses do not undergo accelerated disulfide production. Therefore, it is concluded that the early stage of Emory mouse cataract formation must involve factors other than just accelerated oxidation of protein SH or glutathione SH.

3. No change

4. Not Applicable

5. Publications :

(a) Yu, Nai-Teng, Cai, M.-Z., Ho, D. J.-Y. and Kuck, J. F. R., Jr. (1988)"Automated Laser-Scanning-Microbeam Fluorescence / Raman Image Analysis of Human Lens with Multichannel Detection : Evidence for Metabolic Production of a Green Fluorophor" Proc. Natl. Acad. Sci. USA, *85*, 103-106.

(b) Yu, Nai-Teng and Bursell, S.-E. (1988) "A New Approach to Study Human Cataractogenesis: Fluorescence/Raman Intensity Ratio and Imaging" in Spectroscopic and Structural Studies of Biomaterials I: Proteins(Twardowski, J., Ed.) Sigma Press, Wilmslow, Cheshire, U.K., pp.65-76.

(c) DeNagel, D. C., Bando, Masayasu, Yu, Nai-Teng and Kuck, John F. R., Jr.(1988) "A Raman Study of Disulfide and Sulfhydryl in

the Emory Mouse Cataract" Invest. Ophthalmol. Vis. Sci. *29*, 823-826.

(d) Barron, B. C., Yu, Nai-Teng and Kuck, John F. R., Jr.(1988) "Distribution of a 488.0-nm-excited Fluorophor in the Equatorial Plane of the Human Lens by a Laser Raman Microprobe: A New Concept in Fluorescence Studies" Exp. Eye Res. *47,* 901-904.

(e) Yu, Nai-Teng, Barron, Brent C. and Kuck, John F. R., Jr. (1989) "Distribution of Two Metabolically Related Fluorophors in Human Lens Measured by Laser Microprobe" Exp. Eye Res. (in press).

(f) Yu, Nai-Teng , DeNagel, D. C. and Slingsby, C. (1989) "Raman Spectroscopy of Calf Lens γ-II Crystallin: Direct Evidence for the Formation of Mixed Disulfide Bonds with 2-Mercaptoethanol and Glutathione" Exp. Eye Res. (in press).

(g) Cai, Ming-Zhi, Kuck, John F. R., Jr. and Yu, Nai-Teng (1989) "Galactose-Induced Cataract in Rat: Raman Detection of Sulfhydryl Decrease and Water Increase along an Equatorial Diameter" Exp. Eye Res. (in review).

(h) Barron, Brent C., Yu, Nai-Teng and Kuck, John F.R., Jr. (1988) "Raman Spectroscopic Evaluation of Aging and Long-wave UV Exposure in the Guinea Pig Lens : A Possible Model for Human Aging" Exp. Eye Res. 46, 249-258.